IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#24 Declaration 1/15/03

Attorney Docket Number 040268/0161

In re patent application of
Eric Reynolds

Serial No.: 09/380,738

Filed: December 6, 1999

For: Calcium Phosphopeptide Complexes

Group Art Unit: 1653

Examiner: D. Lukton

Declaration considered - D. L. 2/7/03

DECLARATION UNDER 37 CFR §1.132 OF DR. ERIC REYNOLDS

I, Eric Reynolds of 104 Hill Road, North Balwyn, Victoria 3014, Australia, declare that:

1. I obtained a PhD in 1978 from the University of Melbourne, Australia.

2. I am employed, as Professor of Dental Science and Head of School of Dental Science at The University of Melbourne.

3. I have been doing research and development in the field of Oral Health Science.

4. My curriculum vitae and a list of my publications are appended hereto as EXHIBIT A.

5. I am a sole inventor of US Application Serial Number 09/380,738 ("the application"). I have read a patent record of the application including an Office Action, mailed on July 26, 2002.

6. The application relates to a stable soluble calcium phosphate complex produced by stabilizing amorphous calcium phosphate with phosphopeptide under alkaline conditions. Alkaline conditions can be above pH 7, preferably pH 9.

7. The application reflects a finding that calcium phosphate, in the presence of phosphopeptide, shows different properties or behaviors depending on the pH. As described in the specification, for example, the form of calcium phosphate under acidic conditions, $CaHPO_4$, is known for poor binding affinity to phosphopeptide and poor localization ability at the tooth surface, limiting anticariogenic activity. In contrast, under the alkaline conditions, the amorphous calcium phosphate can be effectively stabilized by phosphopeptide. Thus, the formation of the claimed complex under alkaline conditions in the present application not only prevents from precipitation of calcium phosphate out of its solution but also enhances binding affinity of calcium phosphate to phosphopeptide, thereby providing superior anticariogenic agent with increased calcium bioavailability.

8. The complex, once formed via binding amorphous calcium phosphate to phosphopeptide, has a relatively stable structure where the amorphous calcium phosphate at the core of the structure is shielded from the pH of the solution. That is, an outer layer formed by the phosphopeptide, helps shield the core from salts as well as hydrogen cations and hydroxide anions in solution. As a result, changing pH in a subsequent step does not necessarily affect the structure or properties of the complex formed. The subsequent step may include a step for isolating the complex or a step for formulating the complex with a delivery vehicle such as toothpaste formulation having acidic pH.

9. The structure of the complex, as shown in the accompanying picture, explains the stability of the complex against the change of pH in the surrounding environment. The accompanying picture contains two models of the atomic structure of complexes of the claimed invention. In the models of the complexes, the following color-coding is used: carbon – white, oxygen – red, hydrogen – aqua, phosphate – orange, calcium – purple and nitrogen – blue. I developed these models with my co-workers using a variety of techniques, including in particular two-dimensional NMR. The upper model is a complete model of the complex, showing the atomic structure of the complex according to "space filling" molecular modeling conventions. The lower model is a model of the same complex, but the "space filling" representation of

the structure has been replaced with "stick" models for the outer layer only, to show the "space filling" atomic structure of the core of the complex.

10. Comparison of these two models clearly demonstrates that the outer layer of phosphopeptide effectively shields the calcium and phosphate ions in the center of the complex from hydrogen cations and/or hydroxide anions in an aqueous solution containing the complex. Thus, the pH of the surrounding solution of the complex does not necessarily affect the structure of the complex, which allows the complex to maintain the properties as formed under alkaline conditions, against a subsequent changing of pH in the environment of the complex.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

2/12/02
Date

Dr. Eric Reynolds

CURRICULUM VITAE OF ERIC C. REYNOLDS

Name: Professor Eric C. REYNOLDS
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Present Positions: Head, School of Dental Science, Professor of Dental Science, Director of Research, School of Dental Science, The University of Melbourne.

Most Recent and Highest Academic Qualification: PhD Melbourne 1978.

Awards and Distinctions:

1992 William J. Gies Award presented by the International Association for Dental Research (IADR) and the American Association for Dental Research for the best paper published in Journal of Dental Research

1995 Supervisor of first place Hatton award recipient (pre-doc) Ms C Jackson, IADR meeting Singapore.

1997 Supervisor of second place Hatton award recipient (post-doc) Dr L Huq, IADR meeting Orlando USA.

1997 Alan Docking Science Award presented by the IADR, Australian and New Zealand Division for outstanding scientific achievement in the field of dental research.

1998 Supervisor of second place Hatton award recipient (post-doc) Dr N Slakeski, IADR meeting Nice, France.

2000 Supervisor of Hatton award recipient (post-doc) Dr N O'Brien-Simpson, IADR meeting Washington, USA.

2001 Dairy Industry Association of Australia – The Loftus Hill Memorial Medal Award of Merit – for outstanding scientific achievement.

2002 Clunies Ross National Science and Technology Award to recognize outstanding achievement in the successful application of science and technology for the economic, social and environmental benefit of Australia.

Technology Transfer and Commercialisation:
Inventor and co-inventor of 12 patents and applications (see Patent List in Appendix B). Six of those patents licensed to BFL and Pfizer. Involved in the transfer of that technology to BFL and Pfizer. Closely involved in R&D, product development, regulatory approval and commercialisation of Recaldent™. Six other patents licensed to CSL Ltd.

Selected Publications (1999-2002) *NOTE: Publications restricted by patent applications:*

1. SLAKESKI N, CLEAL SM, BHOGAL PS, **REYNOLDS EC**. Characterization of a *Porphyromonas* gene *prtK* that encodes a lysine-specific cysteine proteinase and three sequence-related adhesins. *Oral Microbiol. Immunol.* 14: 92-97 (1999).
2. PERICH JW, BLACK CL, HUQ NL, **REYNOLDS EC**. Epitope analysis of the multiphosphorylated peptide α_{s1}-casein. *J. Pept. Sci.* 5: 221-233 (1999).
3. **REYNOLDS EC.** Anticariogenic casein phosphopeptides. *Prot. Peptide Lett.* 6: 295-303 (1999).
4. **REYNOLDS EC**, BLACK CL, CAI F, CROSS KJ, EAKINS D, HUQ NL, MORGAN MV, NOWICKI A, PERICH JW, RILEY PF, SHEN P, TALBO G, WEBBER FW. Advances in enamel remineralization: casein phosphopeptide–amorphous calcium phosphate. *J. Clin. Dent.* X: 86-88 (1999).

5. CURTIS MA, KURAMITSU HK, LANTZ M, MACRINA FL, NAKAYAMA K, POTEMPA J, **REYNOLDS EC**, ADUSE-OPOKU J. Molecular genetics and nomenclature of proteases of *Porphyromonas gingivalis*. *J. Periodontal. Res.* 34: 464-472 (1999).
6. HOGAN L, DASHPER SG, MALKOSKI M, TALBO GH, **REYNOLDS EC.** Stimulation of *Porphyromonas gingivalis* Arg-specific proteolytic activity by β-casein(69-93). *Periodontology* 20: 59-66 (1999).
7. **REYNOLDS EC.** The role of phosphopeptides in caries prevention. *Dental Perspectives*. 3: 6-7 (1999).
8. DASHPER SG, **REYNOLDS EC.** The effects of organic acids on growth, glycolysis and intracellular pH of oral streptococci. *J. Dent. Res.*79: 90-96 (2000).
9. O'BRIEN-SIMPSON NM, BLACK CL, BHOGAL PS, CLEAL SM, SLAKESKI N, HIGGINS TJ, **REYNOLDS EC**. Serum immunoglobin G (IgG) and IgG subclass responses to the RgpA-Kgp proteinase-adhesin complex of *Porphyromonas gingivalis* in adult periodontitis. *Infect. Immun.* 68: 2704-2712 (2000).
10. DAWSON NF, CRAIK DJ, McMANUS AM, DASHPER SG, **REYNOLDS EC**, TREGEAR GW, OTVOS L, WADE JD. Chemical synthesis, characterization and activity of RK-1, a novel α-defensin-related peptide. *J. Pept. Sci.* 6: 19-25 (2000).
11. JACKSON CA, HOFFMANN B, SLAKESKI N, CLEAL SM, HENDTLASS AJ, **REYNOLDS EC.** A consensus *Porphyromonas gingivalis* promoter sequence. *FEMS Microbiol. Lett.* 186: 133-138 (2000).
12. HUQ NL, CROSS KJ, **REYNOLDS EC**. Molecular modelling of a multiphosphorylated sequence motif bound to hydroxyapatite surfaces. *J. Mol. Model.* 6: 35-47 (2000).
13. **REYNOLDS EC**, O'BRIEN-SIMPSON NM. Cardiovascular disease and periodontal disease: microbial direct toxic effects on endothelial cells. *Periodontology*. 21: 24-31 (2000).
14. O'BRIEN-SIMPSON NM, PAOLINI RA, **REYNOLDS EC**. RgpA-Kgp peptide-based immunogens provide protection against *Porphyromonas gingivalis* challenge in a murine lesion model. *Infect. Immun.* 68: 4055-4063 (2000).
15. **REYNOLDS EC**. The use of casein phosphopeptides in oral care products for the prevention and treatment of early enamel caries. *Aust. J. Dairy. Technol.* 55: 1-6 (2000).
16. SLAKESKI N, DASHPER SG, COOK P, POON C, **REYNOLDS EC**. A *Porphyromonas gingivalis* genetic locus encoding a heme transport system. *Oral Microbiol. Immunol.* 15: 388-392 (2000).
17. HENDTLASS A, DASHPER SG, **REYNOLDS EC**. Identification of an antigenic protein Pga30 from *Porphyromonas gingivalis* W50. *Oral Microbiol. Immunol.* 15: 383-387 (2000).
18. HUQ NL, CROSS KJ, TALBO GH, RILEY PF, LOGANATHAN A, CROSSLEY MA, PERICH JW, **REYNOLDS, EC**. N-terminal sequence analysis of bovine dentin phosphophoryn after conversion of phosphoseryl to *S*-propylcysteinyl residues. *J. Dent. Res.* 79: 1914-1919 (2000).
19. DASHPER SG, HENDTLASS A, SLAKESKI N, JACKSON C, CROSS K, BROWNFIELD L, HAMILTON R, BARR I, **REYNOLDS EC**. Characterization of a novel outer membrane hemin-binding protein of *Porphyromonas gingivalis*. *J. Bacteriol.* 182: 6456-6462 (2000).
20. PURCELL AW, GORMAN JJ, GARCIA-PEYDRO M, PARADELA A, BURROWS SR, TALBO GH, LAHAM N, PEH AP, **REYNOLDS EC**, LOPEZ DE CASTRO JA, MCCLUSKEY J. Quantitative and qualitative influences of tapasin on the class 1 repertoire. *J. Immunol.* 166: 1016-1027 (2001).
21. DASHPER SG, BROWNFIELD L, SLAKESKI N, ZILM PS, ROGERS AH, **REYNOLDS EC.** Sodium ion-driven serine/threonine transport in *Porphyromonas gingivalis*. *J. Bacteriol.* 183: 4142-4148 (2001).

22. MALKOSKI M, DASHPER SG, O'BRIEN-SIMPSON NM, TALBO GH, MACRIS M, CROSS KJ, **REYNOLDS EC.** Kappacin, a novel antibacterial peptide from bovine milk. *Antimicrob. Agents Chemother.* 45: 2309-2315 (2001).
23. CROSS KJ, HUQ NL, BICKNELL W, **REYNOLDS EC.** Cation –dependent structural features of β-casein(1-25). *Biochem. J.* 356: 277-285 (2001).
24. TALBO GH, SUCKAU D, MALKOSKI M, **REYNOLDS EC**. MALDI-PS-MS analysis of the phosphorylation sites of caseinomacropeptide. *Peptides* 22: 1093-1098 (2001).
25. VEITH PD, TALBO GH, SLAKESKI N, **REYNOLDS EC**. Identification of a novel heterodimeric outer membrane protein of *Porphyromonas gingivalis* by two-dimensional gel electrophoresis and peptide mass fingerprinting. *Eur. J. Biochem.* 268: 4748-4757 (2001).
26. ROSS BC, CZAJKOWSKI L, HOCKING D, MARGETTS M, WEBB E, ROTHEL L, PATTERSON M, AGIUS C, CAMUGLIA S, **REYNOLDS EC,** LITTLEJOHN T, GAETA B, NG A, KUCZEK ES, MATTICK JS, GEARING D, BARR IG. Identification of vaccine candidate antigens from a genomic analysis of *Porphyromonas gingivalis. Vaccine* 19: 4135-4142 (2001).
27. O'BRIEN-SIMPSON NM, PAOLINI RA, HOFFMANN, B, SLAKESKI N, DASHPER SG, **REYNOLDS EC**. Role of RgpA, RgpB and Kgp proteinases in virulence of *Porphyromonas gingivalis* W50 in a murine lesion model. *Infect. Immun* 69: 7527-7534 (2001).
28. SHEN P, CAI F, NOWICKI A, VINCENT J, **REYNOLDS EC**. Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. *J. Dent. Res.* 80: 2066-2070 (2001).
29. VEITH PD, TALBO GH, SLAKESKI N, DASHPER SG, MOORE C, PAOLINI RA, **REYNOLDS EC**. Major outer membrane proteins and proteolytic processing of RgpA and Kgp of *Porphyromonas gingivalis* W50. *Biochem J.* 362: 105-115 (2002)
30. RAJAPAKSE PS, O'BRIEN-SIMPSON NM, SLAKESKI N, HOFFMANN, B, **REYNOLDS EC**. Immunization with the RgpA-Kgp proteinase-adhesin complexes of *Porphyromonas gingivalis* protects against periodontal bone loss in the rat periodontitis model. *Infect Immun.* 70:2480-2486 (2002).
31. SLAKESKI N, MARGETTS M, MOORE C, CZAJKOWSKI L, BARR IG, **REYNOLDS EC.** Characterization and expression of a novel *Porphyromonas gingivalis* outer membrane protein, Omp28. *Oral Microibol. Immunol.* 17:150-156.
32. CHEN YY, CROSS KJ, PAOLINI RA, FIELDING JA, SLAKESKI N, **REYNOLDS EC**. CPG70 is a novel basic metallocarboxypeptidase with C-terminal polycystic kidney disease domains from *Porphyromonas gingivalis*. *J Biol Chem.* 277:23433-23440 (2002).
33. MAZZAOUI SA, BURROW MF, TYAS MJ, DASHPER SG, EAKINS D, **REYNOLDS EC.** Incorporation of casein phosphopeptide-amorphous calcium phosphate into a glass ionomer cement. *J. Dent. Res.* Conditionally accepted for publication December 2001.
34. **REYNOLDS EC.** Health aspects of dairy products –Caries prevention and oral health. Invited Review. In: *Encyclopedia of Dairy Sciences*. Academic Press, California (2002).
35. **REYNOLDS EC**, CAI F, SHEN P, WALKER GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. *J Dent Res.* Accepted for publication November 2002.